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# Antibody biosensors for spoilage yeast detection based on impedance spectroscopy

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## Abstract

*Brettanomyces* is a yeast species responsible for wine and cider spoilage, producing volatile phenols that result in off-odors and loss of fruity sensorial qualities. Current commercial detection methods for these spoilage species are liable to frequent false positives, long culture times and fungal contamination. In this work, an interdigitated (IDE) biosensor was created to detect *Brettanomyces* using immunological reactions and impedance spectroscopy analysis. To promote efficient antibody immobilization on the electrodes' surface and to decrease non-specific adsorption, a Self-Assembled Monolayer (SAM) was developed. An impedance spectroscopy analysis, over four yeast strains, confirmed our device's increased efficacy. Compared to label-free sensors, antibody biosensors showed a higher relative impedance. The results also suggested that these biosensors could be a promising method to monitor some spoilage yeasts, offering an efficient alternative to the laborious and expensive traditional methods.

## Keywords

Biosensor; Impedance Spectroscopy; Spoilage yeasts; Antibody; SAM

## 1. Introduction

*Brettanomyces* is a frequent spoilage yeast found in alcoholic beverages, especially in wine and cider (Chambers and Pretorius, 2010; Claussen, 1904; Silva et al., 2004). These species are responsible for producing metabolic products that are associated with unpleasant flavors and aromas, mostly caused by volatile phenols. During the aging process, *Brettanomyces* can transform natural constituents of both grape and apple juice, p-coumaric and ferrulic acids (hydroxycinnamic acids), into 4-ethyl-phenol and 4-ethyl-guaiacol, respectively (Mansfield et al., 2002; Romano et al., 2009; Rosaria et al., 2015; Steensels et al., 2015). Experts describe the resultant unpleasant aroma, known as "Brett character", as barnyard, mousiness, smoky, or horse sweat (Šućur et al., 2016). This effect causes significant economic losses for beverage industries. Moreover, due to their ability to form biofilms, these species can live for extended periods of time and survive conventional sanitation procedures (Agnolucci et al., 2010; Joseph et al., 2007).

Since microorganismal contamination is a substantial threat to this sector over the last few decades, various detection methods were developed. For *Brettanomyces* detection, different direct or indirect techniques exist, such as plating or molecular analysis, respectively (Wedral et al., 2010). However, these methods present many problems for industries. Plating, a commonly used direct detection method, faces long incubation times and risk of contamination. For

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